Does Egg Coloration Signal Female Quality to House Wren Males (*Troglodytes aedon*)?

Research Thesis

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Abstract:

This experiment sought to clarify a possible correlation between the coloration of female house wren, *Troglodytes aedon*, eggs and an increase in male provisioning. To provide an adequate measure of female quality, information on the female's mass, wing length, laying date, clutch size, and overall brightness of the egg (measured using a portable spectrophotometer) was gathered. Male house wrens feeding rates were also recorded on days 4 and 10 after the first nestling had hatched in order to determine if egg brightness increased their provisioning. A total of 45 nests, along with 139 eggs were sampled in 3 separate locations in northwest Ohio. No relationship was found between the brightness of the egg and the mass and/or wing length of the female house wren. The color of the egg did vary with female laying date but this did not have any correlation with female morphology. However, egg brightness did decline with later laying dates. The feeding rate of male house wrens revealed no significant relationship after controlling for laying date and clutch size. This research provides evidence that female house wren quality does not influence egg brightness or greater male investments.

Introduction:

Pigmentation may be one factor used by many types of birds during mate choice (McGraw et al. 2005). Color in birds is thought to indicate the individual's physical and nutritional condition. For example, male house finches, *Haemorhous mexicanus*, had a lower overall tone of carotenoid-based plumage following a food deprivation (McGraw et al. 2005). Color may also play a role in distinguishing a bird's health. In American goldfinch, *Carduelis tristis*, pigmentation of the plumage is circulated directly through the bloodstream (McGraw et al. 2005). Because color relates to physical condition and health, color could also correlate with reproductive effort. For example, in great tits, *Parus major*, bigger nests were built by females who had larger and brighter breast plumage (Broggi and Senar 2009). In conclusion, color plays a major role in signaling health and potential reproductive contributions among individuals. Besides plumage, egg color is also a trait influenced by natural and sexual selection.

Given that the ancestors of birds likely had white eggs (Kilner 2006), the presence of pigmented shells suggests some evolutionary advantage of pigmentation, such as signaling to others or increasing the eggs' chances of survival. Indication of the validity of this claim comes from the collared flycatcher, *Ficedula albicollis*, a bird which lays a single pale egg last (Krist and Grim 2007). This last laid pale egg is theorized to prevent intraspecific brood parasitism, as the egg would be a signal to other females that incubation of the clutch is already underway (Krist and Grim 2007). Furthermore, the brighter the color of the egg, the more calcium the egg receives (Walters et al. 2014), and the heavier the egg (Walters et al. 2014, Walter and Getty 2010). Lastly, an egg shell's color could adapt to better fit into its surroundings to protect it from predators. Perhaps egg coloration could also have evolved to help better protect the egg from solar radiation (Duval et al. 2012).

Besides natural selection acting on the coloration of the egg to enhance survival, egg coloration could also be a signal between the parents of the egg. The color of the egg might resemble the condition of the female who bore it (Moreno and Osorno 2003). Females with excellent antioxidant systems can contribute more elaborate color into their eggs (Duval et al. 2012). Contrary to this, increased stress could cause eggs to be less colorful (Duval et al. 2012). Pigmentation in egg color could also be costly to produce because already stressed females may have greater trouble depositing larger amounts of color onto their eggs (Walters and Getty 2010). So, females in better quality would likely produce more highly pigmented eggs. Moreover, nestlings that hatched from brighter eggs received higher rates of provisioning from their mothers (Walters and Getty 2010). This could be because females undergoing less stress would prove better at provisioning for their nestlings than other females who were experiencing a greater amount of stress. Therefore, the pigmentation of an egg might give us insight into female condition, such as how much the female weighed, her overall size, clutch size and how early or late she laid her eggs in the spring.

If egg color indicates female quality then a male might provide better parental care to offspring from higher quality females (Moreno and Osorno 2003, Krist and Grim 2007). Blue eggs contain a higher concentration of antioxidants which may be a signal to males to provide better provisioning and other forms of parental investment (Moreno and Osorno 2003). It is theorized that female house wrens, *Troglodytes aedon*, purposely color their eggs brighter to demonstrate quality to males (Walters and Getty 2010). Greater pigmentation in eggs could signal to males a greater female investment in overall egg care (Walters and Getty 2010). For example, male house wrens provided higher provisioning to nestlings when a white egg was experimentally added compared to when a brown egg was added during the incubation stage (Walters et al. 2014). In naturally laid eggs, whiter eggs were heavier than browner eggs (Walters et al. 2014). Males should invest more in provisioning for higher quality nestlings than lower quality nestlings (Walters et al. 2014).

The objective of our study includes two parts. First, we will determine how the color of a female house wren's eggs reflects on her overall quality. We predict that females bearing brighter color eggs (eggs with more white than brown) will have an earlier date of nesting, possess a greater mass and size, and potentially have a greater clutch size compared to other females who have more brown in their eggs. Secondly, we will test the effect that egg color has on the provisioning of the male house wren to nestlings. We predict that a brighter egg would cause the male to provide better provisioning for their nestlings.

Methods:

Study Site:

Our study began in April and ended in August of 2015. A total of 120 nest boxes were spread equally between three different test locations (a wooded area, 40.7363927°N, -84.0266254°W; a golf course 40.752005°N, -84.036931°W; and a park, 40.735647°N, -84.029853°W). The nest boxes that were used all had a depth of 20.3 cm, a width of 10.1 cm, and a length of 14.0 cm. Furthermore, the opening of the box was a circle containing a diameter of 2.9 cm and is located approximately 2.5 cm from the top of the nest box. The small opening excludes most birds, except House wrens.

Study Species:

House wrens are small birds ranging from 11.75-13.5 cm and 10-12 g (Johnson 2014). They are a common species in North America, having two broods per season and 5-10 eggs per clutch. These eggs are small with an average length of 1.66 cm and weighing as much as 1.45 g within the first 6 hours of laying (Johnson 2014). The color of the eggs ranges from white to brown with a mixture of both colors along with some spots of brown among the eggs (Figure 1). The color of the eggs, however, appears to be most heavily concentrated on the larger ends of the egg (Johnson 2014). The nestling period of most house wrens is around 14-18 days. Both the male and female house wren feed the nestlings. These birds are sexually monomorphic and socially monogamous or polygynous (DeMory et al. 2010).

Field Methods:

We checked all the boxes twice a week, to monitor nest construction. When a cup was formed, we checked the box daily to record the exact laying date. We then continued the daily checks until the clutch was complete (female goes two days without laying an additional egg). To test if the egg color of a house wren can indicate greater female quality, we measured the reflectance of the first three eggs that were laid, on the day that the third egg was laid. In the event that the spectrophotometer was not available when the third egg was laid, the reflectance was measured of the entire clutch the next date that it was available. When removing the eggs from the nest, the eggs were placed on cotton for protection. The reflectance of the light and dark ends of the eggs was recorded within 1-m of the nest to prevent the parents' return and discovering an empty nest. We measured the light reflectance from 300-700nm using an Ocean Optics JAZ portable spectrophotometer. The eggs were all measured at a 45° angle. After reflectance was recorded, the eggs were immediately returned to the nest.

We banded both male and female house wrens. We did this by trapping them in the nest box and banding them with a unique combination of colored leg bands on days 4-10 of the nestling period. We measured the mass and wing length (wrist to the most outward feather).

In order to determine if the color of the eggs impacts male provisioning rates, the nest boxes were observed between the hours of 0600-1200 Eastern Daylight Time (EDT) on days 4 and 10 after the hatching of the first nestling. During each 30-minute observations, we kept a tally of how often a male house wren visited the nest. If both the male and the female were unbanded, we used the house wren's behavior to determine the sex if possible. Males typically sing before feeding; whereas, females do not sing and spend significant amounts of time in the box brooding nestlings (typically > 5 minutes, unpublished data). We viewed the nest using Eagle Optics Denali binoculars (8x42) and Zeiss 20-40x85 spotting scopes.

Data Analysis:

To test whether the color of house wren eggs correlates with female quality, we measured the difference in reflectance between the white and dark ends of the egg and the overall brightness of the egg (brightness of white and brown). Additionally, we binned the reflectance measurements into 20nm increments and used a Principle Component Analysis (PC scores) to obtain statistically independent variables of color. We used ANOVAs to determine if female mass or wing length correlates with the color difference or, the overall brightness or PC scores of egg color. In addition, we also used ANOVAs to compare the same metrics of egg color verses male feeding rates. All statistical analyses was conducted in JMP (SAS Institute, Cary, NC).

Results:

We obtained data from 45 nests with a total of 139 eggs. House wren's eggs had a peak of reflectance in the 300-350 and 600-700 portions of the spectrum (Figure 2).

Egg color varied with female laying date but did not relate to female morphology. Egg brightness declined with later laying ($F = 5.03$, $N = 45$ nests, $P = 0.03$; Figure 3). Egg brightness was not related to wing length ($F = 2.45$, $N = 29$, $P = 0.13$) or size-corrected mass ($F = 1.11$, $N = 27$, $P = 0.30$).

The feeding rate did not correlate with the color of the house wren eggs after controlling for laying date and clutch size (Table 1).

Discussion:

We found no relationship between female size and egg coloration. Our study adds to the growing body of literature that found no change in eggshell color, with respect to size or quality of female birds (Honza et al. 2011, Krištofík et al. 2013, and Stoddard et al. 2012). This is relevant to our findings which indicate that female wing length and mass did not influence egg pigmentation, as it demonstrates that a female's physical attributes do not affect egg brightness.

Although the aforementioned studies that support our findings, there are also those that dispute them. In European starlings, *Sturnus vulgaris*, eggshell color did relate to female weight, along with healthier and larger chicks (Fronstin et al. 2016). Other studies have found that eggshell color relates to increased food supplementation (Moreno et al. 2006), the age of the female and body condition (Siefferman et al. 2006), stress levels (Martinez-de la Puente et al. 2007), and female weight (Stoddard et al. 2012). Although we did not find any relationship between eggshell color and female weight that does not mean that there could not have been one. Many factors could have influenced the weight of the house wrens such as location, environment, and consistency of the food supply. We utilized three separate locations, but it is unknown whether each location varies in microhabitat and food supply, and would be a good avenue for future study. Additionally, multiple years of study in recording variation in food supply may be necessary to quantify these confounding factors.

Additionally, the type of pigment used in egg coloration may affect the type of information being communicated through eggshell color. For example, the pigmentation that is responsible for the house wren's brown egg color is called protoporphyrin, while the pigmentation that accounts for the blue-green color in bird species in biliverdin (Hargitai et al. 2016). It has been hypothesized that females depositing biliverdin, containing antioxidant attributes, into their eggs could be costly and signal greater female quality since only high-quality females possess the ability to deposit high levels of biliverdin into their eggs (Moreno and Osorno 2003). In regards to protoporphyrin pigmentation, which contains pro-oxidant compounds, the opposite is true (Hargitai et al. 2016). Poor female conditions and greater stress levels may increase the level of pro-oxidant that is deposited into the egg, thus the darker the pigmentation, the poorer the female condition (Moreno and Osorno 2003, Martinez-de la Puente et al. 2007). This has been shown by Martinez-de la Puente et al. (2007) who concluded that blue tit, *Cyanistes caeruleus*, females with less stress proteins and greater body conditions produced less spotted eggs. Likewise, Stoddard et al. (2012) found that larger great tit, *Parus major*, females produced less speckled eggs, indicating higher quality females were depositing less protoporphyrin into their eggs. Our study, however, did not test for oxidative stress in female house wrens and its relationship to egg coloration. By testing the blood for these properties in female house wrens, one would be able to trace the stress hormones or blood parasites of the house wren and relate that towards her mass and egg coloration.

Natural selection also influences the coloration of eggs. One such study indicated that eggshell color might be hereditary, specifically relating to the female (Sezer and Tekelioglu, 2009). Sezer and Tekelioglu (2009) stated that different eggshell coloration could be due to the selection for crypsis, allowing the eggs to blend in with their nests, thus reducing predation. Coloration could also affect resistance to nest parasites or act as protection from UV light (Sezer and Tekelioglu 2009). It is unknown what elements of natural selection are acting on house wren egg coloration.

We found that the brightness of the egg declined with later laying date. Similarly, Hargitai et al. (2016) found that eggs laid in the clutch possessed greater spot variation and greater blue-green coloration than eggs laid earlier in the canary, *Serinus canaria*. Moreno et al. (2005) and Krist and Grim (2007) further found that females produced eggs that decreased in egg coloration in relation to laying order. Likewise, Dehnard et al. (2015) also showed that in rockhopper penguins, *Eudyptes chrysocome*, the first egg laid had a greater pigmentation of blue-green coloration compared to the second egg that was laid. Contrary to these findings, however, Stoddard et al. (2012) found no relationship between egg color and initial laying date in great tits while Siefferman et al. (2006) actually found an increase in egg coloration with later laying date in eastern bluebirds, *Sialia sialis*. Hanley and Doucet (2009) looked at ring-billed gulls, *Larus delawarensis*, and found that the middle egg possessed the greatest overall blue-green egg coloration. This variation could be a result of environmental factors having an effect on the female. Females experiencing higher levels of stress may cause them to deposit more protoporphyrin into their eggs, causing them to be darker (Hargitai et al. 2016). A female might experience more stress at the beginning of the laying period but as environmental conditions improve, her oxidative stress levels will be reduced.

We also studied parental feeding rate based on egg coloration and found no effect on feeding rate. Like our study, males of many species do not change feeding rates based on egg brightness (Krist and Grim 2007, Lopez-Rull et al. 2007, Walters and Getty 2010, Stoddard et al. 2012). This is contrary to Walters et al. (2014), which found that male house wrens provided higher provisioning to nestlings where a white artificial egg was added. For that study, an artificial egg was placed into the nest and subsequently removed on the first day of hatching (Walters et al. 2014). Unfortunately, the artificial eggs also possessed a greater brightness than those found in naturally occurring eggs (Walters et al. 2014). Male house wrens might not provide higher provisions to eggs that were naturally lighter or naturally brighter eggs might not make enough of a difference to cause higher male provisioning.

A study done by Morales et al. (2010) is more supportive of the relationship between egg coloration and male provisioning. Although they came to the same conclusions as we did, they found that greater egg pigmentation resulted in greater parental sharing of incubation (Morales et al. 2010). This could be due to females needing to rest more frequently after providing more pigmentation to the eggs and thus requiring more help (Morales et al. 2010). Male wrens do not participate in incubation, but perhaps the proportional changes in feeding rates between the sexes could be examined.

Our results show female quality does not affect egg coloration in house wrens, but other attributes such as laying order, genetic traits, stress levels, or parasite infections were not measured. Furthermore, we found evidence to suggest that egg brightness decreases with laying date. We suggest that food availability may be one factor mediating this relationship.

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Literature Cited

Broggi, J., and Senar, C. J. 2009. "Brighter Great Tit Parents Build Bigger Nests." *International Journal of Avian Science* 151.3: 588-591.

Dehnhard, N., Pinxten, R., Demongin, L., Camp, J. V., Eens, M., and Poisbleau, M. 2015. "Relationships Between Female Quality, Egg Mass and Eggshell Blue-Green Colouration in Southern Rockhopper Penguins: A Test of the Sexual Signalling Hypothesis." *Polar Biology* 38.11: 1805-811.

DeMory, M. L., Thompson, F. C., and Sakaluk, K. S. 2010. "Male Quality Influences Male Provisioning In House Wrens Independently of Attractiveness." *Behavioral Ecology* 21.6: 1156-1164.

Duval, C., Cassey, P., Miksik, I., Reynolds, J. S., and Spencer, A. K. 2012. "Condition-Dependent Strategies of Eggshell Pigmentation: An Experiment Study of Japanese Quail (*Coturnix japonica*)." *The Journal of Experimental Biology* 216.4: 700-708.

Fronstin, B. R., Doucet, M. S., and Christians, K. J. 2016. "Haematocrit, Eggshell Colouration and Sexual Signaling in the European Starling (*Sturnus vulgaris*)." *BMC Ecology* 16.31.

Hanley, D., and Doucet, S. M. 2009. "Egg Coloration in Ring-Billed Gulls (*Larus delawarensis*): A Test of the Sexual Signaling Hypothesis." *Behavioral Ecology and Sociobiology* 63.5: 719–729.

Hargitai, R., Boross, N., Nyiri, Z., and Eke, Z. 2016. "Biliverdin-and Protoporphyrin-Based Eggshell Pigmentation in Relation to Antioxidant Supplementation, Female Characteristics, and Egg Traits in the Canary (*Serinus canaria*)." *Behavioral Ecology and Sociobiology* 70.12: 2093-2110.

Honza, M., Požgayová M., Procházka P., and Cherry I. M. 2011. “Blue-Green Eggshell Coloration Is Not a Sexually Selected Signal of Female Quality in an Open-Nesting Polygynous Passerine.” *Naturwissenschaften* 98.6: 493–499.

Johnson, L. S. 2014. “House wren (*Troglodytes aedon*), version 2.0.” The Birds of North America Online (P. G. Rodewald, editor). Ithaca: *Cornell Lab of Ornithology*; Retrieved from the Birds of North America Online.

Kilner, R. M. 2006. “The Evolution of Egg Colour and Patterning in Birds.” *Biological Reviews of the Cambridge Philosophical Society* 81.3: 383–406.

Krištofík, J., Darolová, A., Griggio, M., Majtán, J., Okuliarová, M., Zeman, M., Zídková, L., and Hoi, H. 2013. "Does Egg Colouration Signal Female and Egg Quality in Reed Warbler (*Acrocephalus scirpaceus*)?" *Ethology Ecology & Evolution* 25.2: 129-43.

Krist, M., and Grim, T. 2007. “Are Blue Eggs a Sexually Selected Signal of Female Collared Flycatchers? A Cross-Fostering Experiment.” *Behavioral Ecology and Sociobiology* 61.6: 863-876.

Lopez-Rull, I., Cells, P. and Gil, D. 2007. “Egg Colour Covaries With Female Expression of a Male Ornament in the Spotless Starling (*Sturnus unicolor*).” *Ethology* 113.10: 926–933.

Martínez-de la Puente, J., Merinon S., Moreno J., Tomás G., Morales J., Lobato E., García-Fraile, S., and Martínez, J. 2007. “Are Eggshell Spottiness and Colour Indicators of Health and Condition in Blue Tits *Cyanistes caeruleus*?” *Journal of Avian Biology* 38.3: 377–384.

Mcgraw, K. J., Hill, E. G., and Parker, S. R. 2005. "The Physiological Costs of Being Colourful: Nutritional Control of Carotenoid Utilization in the American Goldfinch (*Carduelis tristis)*." *Animal Behaviour* 69.3: 653-660.

Morales, J., Roxana T., and Velando, A. 2010. "Parental Conflict and Blue Egg Coloration in a Seabird." *Naturwissenschaften* 97.2: 173-180.

Moreno J., Lobato E., Morales, J., Merino, S., Tomás, G., Martínez-de la Puente, J., Sanz, J. J., Mateo, R., and Soler, J. J. 2006. "Experimental Evidence That Egg Color Indicates Female Condition at Laying in a Songbird." *Behavioral Ecology* 17.4: 651–655.

Moreno, J., and Osorno, L. J. 2003. "Avian Egg Colour and Sexual Selection: Does Eggshell Pigmentation Reflect Female Condition and Genetic Quality?" *Ecology Letters* 6.9: 803-806.

Moreno, J., Morales J., Lobato, E., Merino S., Tomás G., Martínez-de la Puente, J. 2005. "Evidence for the Signaling Function of Egg Color in the Pied Flycatcher *Ficedula hypleuca*." *Behavioral Ecology* 16.5: 931–937.

Sezer, M., and Tekelioglu, O. 2009 "Quantification of Japanese Quail Eggshell Colour by Image Analysis." *Biological Research* 42.1: 99-105.

Siefferman, L., Navara, K. J., and Hill G. E. 2006. "Egg Coloration is Correlated With Female Condition in Eastern Bluebirds (*Sialia sialis*)." *Behavioral Ecology and Sociobiology* 59.5: 651–656.

Stoddard, M. C., Fayet, L. A., Kilner, M. R., and Hinde, A. C. 2012. "Egg Speckling Patterns Do Not Advertise Offspring Quality or Influence Male Provisioning in Great Tits." *PLoS ONE* 7.7.

Walters, L. A., Olszewski, N., and Sobol, K. 2014. "Male House Wrens Provide More Parental Provisioning to Nests With a Brighter Artificial Egg." *The Wilson Journal of Ornithology* 126.3: 508-515.

Walters, L. A., and Getty, T. 2010. “Are Brighter Eggs Better? Egg Color and Parental Investment by House Wrens.” *Journal of Field Ornithology* 81.2: 155-166.

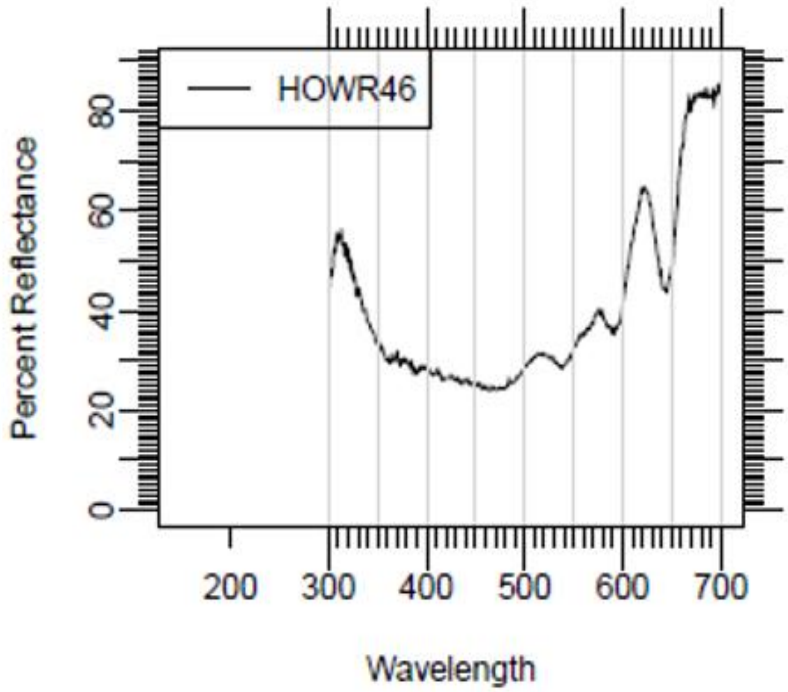
Table 1: Results of an ANOVA comparing how female and male visits to the nest relates to egg brightness, laying date, and clutch size.

Response Variable	Egg Brightness	Laying Date	Clutch Size	Overall Model
4 Day Total Visits	F=0.35, P=0.56	F=1.40, P=0.25	F=4.50, P=0.04	$F_{3.30}$=2.71, P=0.06
4 Day Female Visits	F=0.04, P=0.84	F=2.58, P=0.12	F=1.92, P=0.18	$F_{3.30}$=1.84, P=0.16
4 Day Male Visits	F=0.76, P=0.39	F=0.40, P=0.53	F=1.87, P=0.18	$F_{3,30}$=1.46, P=0.24
10 Day Total Visits	F=1.64, P=0.21	F=5.67, P=0.02	F=2.16, P=0.15	$F_{3,31}$=2.59, P=0.07
10 Day Female Visits	F=0.78, P=0.38	F=5.02, P=0.03	F=0.37, P=0.55	$F_{3,31}$=1.78, P=0.17
10 Day Male visits	F=0.01, P=0.91	F=0.01, P=0.91	F=0.18, P=0.67	$F_{3,31}$=0.06, P=0.98

Figure 1: Image of a nest of house wren eggs

Figure 2: Spectrograph of the end of a house wren egg, dark side (A), and the light side (B). Note that the scale varies on the Y-axis.

A



B

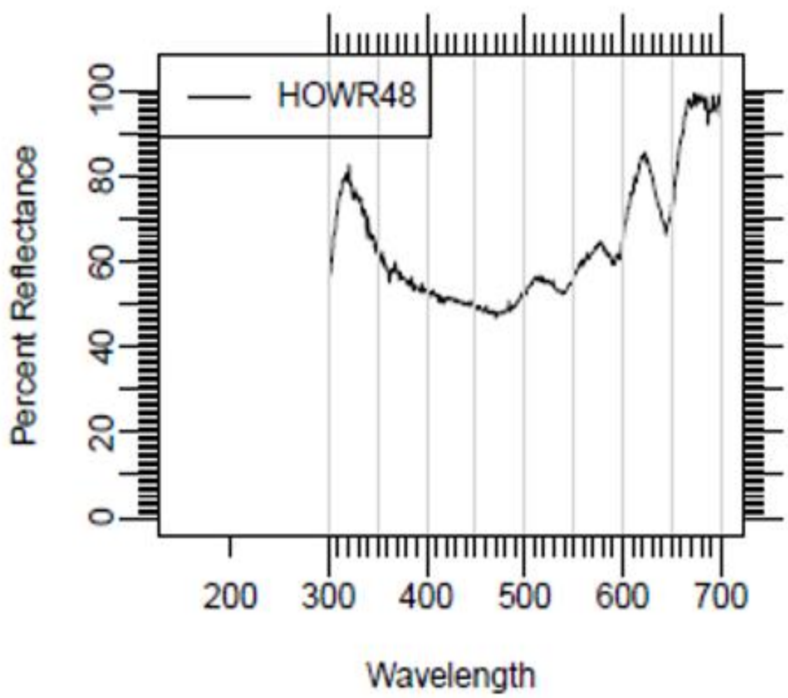


Figure 3: The brightness of house wren eggs declined with laying date.